Summary

This thesis describes the *in vivo* (entrapped cells) and the *in vitro* (enzyme preparations) production of catechols using cell cultures of *Mucuna pruriens* L.. The bioconversion spectrum was studied and kinetic parameters were determined.

In *Chapter 1* the current state of plant cell biotechnology and the possibilities to produce valuable compounds by means of biosynthesis and bioconversion are discussed.

In *Chapter 2* the methods of analysis, used throughout the studies, are presented. A general isolation procedure for catechols is described. Sample purification was performed on Sephadex G 10 and catechols were concentrated on Affigel 601, resulting in samples, free of proteins, polysaccharides and electrolytes, and directly suitable for identification by mass spectrometric analysis. A new technique, combined liquid chromatography / mass spectrometry (LC/MS) is presented. This method offers the advantage to omit derivatization, as necessary for GC/MS and to monitor the mass spectra of compounds which can be seperated with HPLC, on line. To allow an accurate kinetic comparison between *in vivo* an *in vitro* bioconversion, a protein assay was adapted for freely suspended and entrapped cells, as well as for enzyme preparations.

The bioconversion of L-tyrosine into L-DOPA was used for kinetic studies with cells of *M. pruriens* entrapped in calcium alginate, calcium pectinate, agarose or gelatine *(Chapter 3)*. In all cases L-DOPA was released directly into the incubation medium and on the basis of initial rates of bioconversion Michaelis-Menten kinetics was applied. For all matrices, the apparent affinity constants were comparable with the affinity constants obtained with enzyme preparations. Comparison of the apparent maximum rate of bioconversion of the entrapped cells and the maximum rate of bioconversion of a cell homogenate, indicated that the plant cell systems did not operate optimally.

Measurement of the effective diffusivities of L-tyrosine with empty beads showed that this substrate could diffuse freely into the matrices. From the calculated observable moduli it was concluded that the diffusional supply rate of L-tyrosine was not the limiting factor. For oxygen, used by both bioconversion and cell respiration, the calculated moduli indicated strong limitations. Based on the highest cell viability and the highest rates of bioconversion, alginate entrapped cells of *M. pruriens* formed the best biocatalytic system for the production of L-DOPA from L-tyrosine.

Alginate entrapped cells of *M. pruriens* were used for further investigations on the production of catechols. It was shown that 14 *para*-substituted monophenols, including several not naturally occurring compounds could be converted into the corresponding

catechols (*Chapter 4*). Analysis of the bioconversions demonstrated that the bioconversion spectra of the entrapped cells and of enzyme preparations were identical. All produced catechols were released into the incubation medium, allowing efficient isolations. The identity of the products was confirmed by LC/MS or by the desorption chemical ionization technique, depending on the catechol.

For further kinetic studies of alginate entrapped cells of *M. pruriens*, the bioconversion of tyramine, *para*-hydroxyphenylpropionic acid, *para*-hydroxyphenylacetic acid and L-tyrosine were chosen (*Chapter 5*). The apparent affinity constants were comparable with the affinity constants obtained with phenoloxidase preparations. The apparent maximum rates of bioconversion of the entrapped cells were about 50% of the maximum rates of a cell homogenate. This indicates that the cell system did not operate optimally.

The effective diffusivities of the substrates and the products were measured with alginate entrapped, inactivated cells. From five inactivation methods tested, glutaric aldehyde treatment appeared to be relatively the best method. The effective diffusivities found for the monophenols and catechols demonstrated that these compounds were able to diffuse freely into and out of the beads. The calculated observable moduli for the four monophenolic substrates indicated that the diffusional supply rates were not the limiting factor, but for the *ortho*-hydroxylation of tyramine a value higher than 1 was found. From the moduli calculated for oxygen, it was concluded that there were strong oxygen limitations for each bioconversion.

Two additional system parameters were investigated. By varying the cell aggregate size for entrapment, it was demonstrated that the bioconversion rates were dependent on the degree of cell-matrix contact. By varying the charge of beads, it was shown that the mixing conditions chosen for our kinetic studies were sufficient.

The low substrate specificity of alginate entrapped cells of *M. pruriens* opens perspectives for the *ortho*-hydroxylation of substrates with more complex chemical structures. Aminotetralin derivatives and related compounds were successfully subjected to *in vivo* and *in vitro* bioconversion (*Chapter 6*). The 5-, 6- and 7-hydroxy-aminotetralins and a tricyclic substrate, 9-hydroxy N-n-propyl hexahydronaphthoxazine were converted into catechols. For all bioconversions with the entrapped cells the release of catechols was observed. In general, the bioconversion products were separated and isolated by preparative HPLC using a volatile acetate buffer. The identity of the products was confirmed by the desorption chemical ionization technique.

Finally, a continuous flow system for the production of 7,8-dihydroxy N-di-n-propyl aminotetralin was developed. The system consisted of a *Mucuna*-phenoloxidase suspension in dialysis tubing as the biocatalysator in an airlift-fermentor and an aluminium oxide column for continuous selective product isolation.

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The final chapter (*7*) deals with general aspects concerning the production of catechols by *in vivo* and *in vitro* bioconversion. In order to enhance the bioconversion efficiency of alginate entrapped cells of *M. pruriens*, the freely suspended cells were subjected to two procedures focussed on the selection of cells with high phenoloxidase activity. The procedures based on selection for the green colour as a criterion for the presence of chloroplasts, and for fluoro-tyrosine resistance were not successful.

From a comparison of phenoloxidase activities in cells of *M. pruriens* with those in cells derived from non-L-DOPA accumulating plant species, it was concluded that suspension grown cells of *M. pruriens* were the best source for enzyme activity.

Finally, suggestions are made with respect to an optimal use of the phenoloxidase activity *in vivo* or *in vitro* and aspects concerning the production of valuable compounds by plant cells and derived enzyme preparations are discussed.